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Abstract

Climate models predict increases in frequency and intensity of extreme environmental conditions, such as changes to minimum and maximum temperatures, duration of drought periods, intensity of rainfall/snowfall events, and wind strength. These local extremes, rather than average climatic conditions, are closely linked to woody plant survival, as trees cope with such events over long lifespans. While xylem provides trees with structural strength and is considered the most robust part of a tree's structure, it is also the most physiologically vulnerable since tree survival depends on its ability to sustain water supply to the tree crown under variable environmental conditions. Many structural, functional, and biological tree properties evolved to protect xylem from loss of transport function due to embolism or to restore xylem transport capacity following embolism formation. How 'the new climate normal' conditions will affect these evolved strategies is yet to be seen. Our understanding of xylem physiology and current conceptual models describing embolism formation and plant recovery from water stress, however, can provide insight into near future challenges that woody plants will face. In addition, knowledge of species-specific properties of xylem function may help guide mitigation of climate change impacts on woody plants in natural and agricultural tree communities.

Introduction

Trees are long-lived organisms, anchored in one place for tens, hundreds, or thousands of years. They rely on a vascular transport system that, although continuously renewed via annual xylem and phloem growth, must endure local climatic extremes including events of variable frequency, intensity, and duration. Global climate models predict that the small changes in recent climate observed across the planet are only a prelude to more dramatic changes in the near future, such as prolonged drought periods and increased frequency of frost events (IPCC, 2013, (Bloom, 2010, Hayhoe *et al.*, 2004). Changing frequency or intensity of extreme events might overwhelm the adaptive limits of vascular transport, resulting in continued periods of vascular function loss and increased incidence of tree mortality. In fact, we already observe increased mortality of trees in natural ecosystems stretching across multiple regions around the world that are being linked to drought and carbohydrate starvation (Hartmann *et al.*, 2013, O'Grady *et al.*, 2013, Sevanto *et al.*, 2014). Not only are natural ecosystems in danger, the same fate might be expected for the perennial woody crop plants in orchards. Despite increased agro-engineering efforts, we are limited in our ability to protect orchard trees from global scale consequences of climate change, including loss of access to fresh water, increased salinization of agricultural soils, and rising atmospheric temperature. In addition, orchards are planted today with the expectation of continued productivity for the next 20-40 years, an expectation that might be increasingly difficult to achieve under predicted climate change scenarios (Lobell *et al.*, 2006). To mediate potential damage to agricultural investments and protect natural forest ecosystems, we should better understand how “new climate normal” conditions will influence critical aspects of vascular physiology, namely vascular embolism formation and recovery.

Embolism

Long-distance water transport in vascular plants occurs in the xylem, a network of nonliving cells connecting roots to leaves (Sperry *et al.*, 2003). Typically, the apoplastic water column in functional xylem is under tension and considered to be in metastable state (Stroock *et al.*, 2014). When internal tension reaches a critical threshold at which the conduit radius is larger than the critical radius of the vacuum/water interface (critical radius $r_{critical}$ being defined as equality between meniscus force defined as water surface tension (γ) divided by air bubble radius) that could oppose expansion due to water surface tension, the conduit water column can break forming an embolus, a phenomenon referred by many plant biologists as 'cavitation' (Tyree & Zimmermann, 2002). We note that cavitation (considered as spontaneous initiation of vapor bubbles in pure metastable water) is a highly improbable event in xylem, as it occurs spontaneously at pressures far more negative [i.e. below -140 MPa (Brenner, 1995)] than are physiologically relevant (i.e. above -20 MPa). Thus, we will abstain from using word 'cavitation' and only refer to the process as 'embolization' or 'formation of embolism'. Despite the very low probability of true spontaneous cavitation in stems of trees, it was shown using multiple indirect and direct methods that xylem conduits (vessels and tracheids) can switch from a water filled to an embolized state even under tension as low as 1 MPa (Brodersen *et al.*, 2010, Holbrook *et al.*, 2001, Zwieniecki *et al.*, 2013). To reconcile the low probability of spontaneous cavitation with observations of naturally occurring embolism levels, an "air-seeding" hypothesis was proposed as a possible mechanism to explain formation of embolism in xylem under relatively low tensions (Zimmermann, 1983).

"Air-seeding" is assumed to have several potential origins that are not mutually exclusive, all of which can influence the level and threshold for xylem embolism formation (Capron *et al.*, 2014, Zimmermann, 1983). All proposed explanations assume that somewhere along the conduit an air-water interface exists with a radius that is smaller than the expansion threshold (Figure 1). This interface can exist across the inter-vessel connection (bordered pit field) (Lens *et al.*, 2013), in the crevices of the vessels or tracheid (Pickard, 1981, Zimmermann, 1983), or simply on the wall surface where stable bubbles persist for a prolonged time (Weijs & Lohse, 2013). Increasing tension would result in the change of the critical radius required to overcome water surface tension such that the bubble slowly changes shape and expands into the conduit eventually reaching a critical radius and 'explodes' into the conduit causing an embolism that effectively ceases water transport as tension cannot be transmitted via gas. This type of embolism formation might be considered a spatially and temporally unpredictable phenomenon or it might be related to the degree of tension, thermal environment, xylem physical properties, water chemical properties, and history of previous embolism activity (Hacke *et al.*, 2001, Holbrook & Zwieniecki, 1999, Stiller & Sperry, 2002, Tyree & Zimmermann, 2002). In addition to purely tension driven embolism, another origin of the embolism results from freeze-thaw events, where ice formation may lead to release of air from water which form small gas bubbles in the frozen liquid (Mayr *et al.*, 2007, Sperry & Sullivan, 1992). The fate of air bubbles during thawing depends on their size and the amount of tension in the xylem during thawing. If the air bubble radius is smaller than the critical radius for expansion and pressure differential between air in the bubble and water ($2\gamma/r_{critical}=P_{air}-P_{water}$), the air will dissolve in water and the bubble will either collapse or attach to the conduit wall forming a future "air-seeding" site. Alternatively, if

99 the air bubble radius is larger than the critical radius, the bubble will expand and an embolus will
100 form.
101 Regardless of embolism origin, the result is a reduction in the plant's capacity to transport water
102 and a loss of plant productivity or even plant death (Domec *et al.*, 2006b, Holtta *et al.*, 2009,
103 Tyree & Sperry, 1989, Zwieniecki & Holbrook, 2009). Therefore, a plant's capacity to reduce
104 the detrimental effects of embolism is an important adaptive trait for growth and survival
105 (Barigah *et al.*, 2013, Choat *et al.*, 2012, Jacobsen *et al.*, 2007, Pockman *et al.*, 1995, Tyree &
106 Ewers, 1991) and can be affected by climatic properties, especially temperature and moisture.
107 Resistance to drought and frost-induced embolism are important adaptive traits for defining the
108 limits of embolism tolerance across woody species and for predicting drought-induced forest
109 decline at regional and global scales (Choat *et al.*, 2012, Lens *et al.*, 2013). To better understand
110 the role of climate in this system we need to look at the anatomy and associated physicochemical
111 properties of xylem including (i) the structure of the pit membranes, (ii) diameter and length of
112 conduits and (iii) wood density within the context of major climate variables.
113
114 *Pit membranes.*
115
116 Bordered pits are cavities in the lignified cell walls of adjacent vessel or tracheid conduits. They
117 are a pivotal component in the water-transport system of seed plants. Bordered pits are not
118 simple holes that allow water passage between cells. They are highly engineered structures that
119 are strongly conserved among species and that are responsible for more than 50% of total xylem
120 hydraulic resistance (Choat *et al.*, 2008, Hacke *et al.*, 2006, Wheeler *et al.*, 2005) indicating that
121 they are an important factor in the overall hydraulic efficiency of plants and may be fundamental

to species survival. In general, bordered pits are formed as circular openings with species-specific diameters ranging from 1 to 20 µm, protected on both ends by a bowl shaped wall, typically with a smaller opening leading to the pit. The pit membrane is located at the center where it separates adjacent conduits and mediates two seemingly contradictory functions: (1) allowing water to flow between adjacent conduits and (2) protecting conduits against the spread of embolism (Choat *et al.*, 2008, Tyree & Sperry, 1989).

The structure of pits and pit membranes varies among species, with differences in pit diameter, pit depth, wall smoothness, membrane porosity, membrane thickness, and membrane total surface area. For example, among 26 angiosperm species collected from a range of provenances including riparian, temperate, and Mediterranean environments, the pit membrane thickness range from 70 to 1900 nm and pore sizes within the membrane from 10 to 225 nm (Jansen *et al.*, 2008). Species characterized by thicker pit membranes usually also have smaller pores and thus are better adapted to protection from embolism spread as radius of the pores is inversely related to critical tension sustained at the air/water interface. Jansen et al. (2008) also found a negative relationship between pit membrane thickness and maximum pore diameter. In addition, pore size in a given species is variable with species specific probability of large pores. As larger pores allow embolism to spread more easily from conduit to conduit, the species with bigger pores or with higher probability of their presence due to a larger membrane area between conduits will be more vulnerable to vascular dysfunction (Choat *et al.*, 2008). Interestingly, there was no correlation between hydraulic resistance of the pit membrane and vulnerability to embolism across a wide range of species. Species with lower hydraulic resistance and higher average porosity were not necessarily more vulnerable to embolism (Hacke *et al.*, 2006). This discrepancy could be explained by the fact that it takes only one large pore in one membrane to

spread embolism from conduit to conduit. As such big pores are relatively rare and might be considered a membrane developmental error or could be a result of damage (Choat, 2013). Increased cavitation risk may therefore be linked to total pitted area of xylem conduits, making vessels with greater total area of pits more vulnerable to embolism (Jarbeau *et al.*, 1995). In most gymnosperms, the bordered pit membrane is characterized by a porous margo and central thickening (torus), which is considered to function as an impermeable safety valve against air-seeding. Embolism resistance of conifer pits is correlated to the ratio of torus size versus pit aperture diameter, suggesting a dependency on the torus' ability to seal the pit aperture (Domec *et al.*, 2006a). Embolism resistant species showed higher margo flexibility and larger overlap between the torus and pit aperture, suggesting that torus adhesion to the pit border may be a determinant of cavitation resistance (Delzon *et al.*, 2010). In some conifer species the presence of plasmodesmatal pores fields in the tori could contribute to air-seeding through them (Jansen *et al.*, 2012) reducing effectiveness of the pore seal.

Overall, bordered pit membranes whether in gymnosperms or angiosperm trees seem to be the first line of defense against conduit-to-conduit spread of embolism. A plasticity of bordered pit morphology related to embolism formation was reported for Douglas-fir trees. It was shown that in the branches and trunks the pit aperture diameter of tracheids decreases significantly with increasing height, whereas torus diameter remains unchanged. The resulting increase in the ratio of torus to pit aperture diameter provides better overlap of torus with pit aperture, resulting in the capacity to sustain higher tensions before air-seeding (Domec *et al.*, 2008). There is also a general pattern of smaller pits with thicker, less porous pit membranes in trees growing in drier climates. However, almost nothing is known regarding the plasticity of bordered pit

morphological properties over the lifespan of a tree and whether the plant can respond to climatic change. Specifically, it is not known if drier years trigger development of thicker pit membranes and/or smaller pit areas within the same tree across multiple rings, ultimately leading to reduced membrane porosity. This lack of knowledge leaves us unable to predict if trees growing today can acclimate to changes in climate via developmental adjustment of bordered pit structure. However, assuming that bordered pits evolved to protect xylem from embolism spread during extreme events in the current climate, it is conceivable that increased frequency of extreme weather events with more adverse conditions might lead to higher frequency of bordered pit failures and greater incidence of embolism formation.

Diameter and length of conduits

Angiosperm and gymnosperm conducting elements differ in both diameter and length. Vessels are bigger (length: 1-1000+ cm, diameter: 15-500 μm) than tracheids (length: 0.1-1.0 cm, diameter: 5-80 μm) although the specific distribution of vessel lengths is not known (Choat *et al.*, 2008, Zimmermann, 1983). Multiple lines of evidence suggest that wide and long conduits tend to embolize before narrow conduits (Lo Gullo & Salleo, 1991, Lo Gullo *et al.*, 1995, Sperry & Tyree, 1990). This may simply be related to the fact that conduits may contain more bordered pit pores with larger total pit membrane area and thus higher probability of embolism spread due to damage, malfunction, or developmental error (pit area hypothesis) (Sperry *et al.*, 2005, Tyree *et al.*, 1994, Wheeler *et al.*, 2005). Indeed a strong correlation was found between conduit diameter and embolism caused by freeze-thaw cycles (Davis *et al.*, 1999). However, analysis of anatomical traits of xylem plasticity to water stress is more complex (Tyree *et al.*, 1994) as trees

were shown to plastically adjust their xylem anatomical traits (diameter and length of the conduits) to drought. These adjustments are not straightforward as plants have to accommodate contradicting demands, namely transport capacity and resistance to embolism formation. For example, *Pinus sylvestris* trees grown in dry regions were characterized by large tracheid lumens, thicker cell walls and high ray tracheid frequency. All of these anatomical features are suggested to facilitate efficient water transport and stem water storage (Martin *et al.*, 2010), but potentially lead to reduced protection from embolism spread between tracheids (pit area hypothesis). In addition, the simple notion that conduit diameter relates to embolism formation is further complicated by the fundamental difference between gymnosperms and angiosperms. Gymnosperms have unicellular tracheids that are a few millimeters long, whereas angiosperms have multicellular vessels with lengths measured in centimeters to meters. This anatomical diversity is most likely responsible for the lack of a simple relationship between conduit diameter and embolism pressure across tracheids and vessels (Sperry *et al.*, 2006).

These anatomical trade-offs can be considered in light of expected climatic change, although specific predictions are hard to make. In general, drier weather patterns would promote formation of narrower vessels in angiosperm species, a tendency that would limit the probability of embolism formation. This, however, would lead to the reduction of xylem transport capacity, potentially increasing hydraulic resistance and internal tension, thus, eventually leading to zero or negative net effect of this strategy to avoid embolism. Although xylem structure can acclimate to environmental variation during growth and development (Maherali & DeLucia, 2000a, Maherali & DeLucia, 2000b, Thomas *et al.*, 2007), the subsequent acclimation of embolism resistance to stress is not possible because xylem conduits are dead at maturity (Choat

et al., 2012). Thus, the presence of extreme weather events may further undermine this anatomical adaptation strategy leading to embolism related dieback of already established trees. At the very least, the tendency to grow narrower xylem conduits would most likely result in less efficient transport system and would lead to overall reduction in photosynthetic capacity and reduced growth rates.

Wood density

Wood density is another morphological parameter often related to a tree's ability to withstand embolism. It depends on cellular wall thicknesses of tracheid and vessels, lumen diameters, and percentage of latewood (part of the wood in a growth ring of a tree that is produced later in the growing season). It was suggested that wood density and specifically wall thickness is related to the risk of cell wall collapse under tension and embolism due to mechanical cell damage (Jacobsen *et al.*, 2005, Sperry *et al.*, 2003, Wagner *et al.*, 1998). Indeed, species characterized by a high wood density and a high thickness-to-span ratio [thickness:span represents the ratio of conduit double wall thickness to lumen diameter, (Pittermann *et al.*, 2006)] of water conducting cells are more resistant to embolism formation (Hacke *et al.*, 2001) and trees producing low-density wood under favorable water availability are more stressed by sudden drought (Rosner *et al.*, 2014).

It is very unlikely that cellular walls directly contribute to embolism spread between conduits. Wall forming cellulose fibers are impregnated with lignin, hemicelluloses and pectin limiting potential radius of pores to be smaller than those existing in bordered pit membranes. However,

under changing climatic conditions, induction of drought response might lead to changes in wall surface chemistry (Kostiainen *et al.*, 2006). Lignin is a hydrophobic component of the xylem conduits walls. Thus one can speculate that changes in lignin wall content in response to water stress would be responsible for the increase of wall hydrophobicity. Consequently, the surface would be more likely to nest persistent air pockets responsible for 'air seeding', thus limiting the effectiveness of this response as an embolism avoidance strategy. Similarly, genetic manipulation aiming at decreased lignin xylem content for fuel production might also have unintended consequences, as a reduction in lignin content generally leads to thinner walls and increases the potential of conduit collapse under tension (Donaldson, 2002) and higher vulnerability to embolism (Voelker *et al.*, 2011). Again, we are left with highly limited knowledge of natural potential for acclimation of trees to changing climate via adaptation of wood structure.

Tree mortality - hydraulic failure

In the past 20 years an increased frequency of widespread forest mortality due to drought or temperature stress has been observed in many different tree-dominated ecosystems, such as tropical rainforest (Phillips *et al.*, 2009), temperate mountainous forests, Mediterranean forests, and boreal forests (Carnicer *et al.*, 2011, Peng *et al.*, 2011, van Mantgem *et al.*, 2009). Also, in temperate North American forests some mortality events were associated with "global change-type droughts", defined as severe drought coupled with elevated summer temperatures (Breshears *et al.*, 2005, Shaw *et al.*, 2005, Worrall *et al.*, 2010). Therefore, to make predictions of how future climate change scenarios will affect different biomes it is critical to understand the

physiological mechanisms responsible for tree mortality. In this context, we have to consider (i) hydraulic failure and (ii) carbon starvation (McDowell *et al.*, 2008, Nikinmaa *et al.*, 2013, Sevanto *et al.*, 2014).

Overall, evolution of xylem conduits can be described as an increase in transport efficiency at the expense of embolism protection and vice versa. Trees dominate multiple ecosystems that are characterized by high moisture availability. However, despite over 350 million years of evolution, trees have a limited presence in many drier biomes. Woody plants mostly exist as shrubs that deal with stress related injuries by re-growing lost stems. The absence of trees in dry habitats suggests their limited ability to form a transport system that is perfectly protected from embolism under extreme drought conditions. In areas occupied by trees, they may experience 'hydraulic failure' when water loss from transpiration is greater than water uptake by roots, creating high negative xylem tension that may result in embolism (Sevanto *et al.*, 2014, Sperry *et al.*, 1998).

The ability of a plant to protect itself from hydraulic failure is often described in terms of its 'hydraulic safety margin' (Meinzer *et al.*, 2009). This trait is often analyzed using 'vulnerability curves' that plot how xylem hydraulic conductivity (Kh) declines as xylem pressure becomes more negative. Declines in Kh are typically expressed relative to the maximum sample Kh as the percentage loss of conductivity (PLC). Such curves are typically sigmoidal in shape with low PLC at xylem pressure near zero and high PLC at large negative pressures. Embolism resistance has been widely documented across species and varies greatly (Maherali *et al.*, 2004). There is also evidence that embolism resistance can vary between populations of the same species (Alder

283 *et al.*, 1996, Mencuccini & Comstock, 1997, Sparks & Black, 1999) or between closely related
284 genotypes (Barnard *et al.*, 2011, Choat *et al.*, 2007, Neufeld *et al.*, 1992, Pita *et al.*, 2003,
285 Sangsing *et al.*, 2004). Usually, comparisons of vulnerability to embolism among species are
286 represented by the xylem pressure at which a 50% loss of conductivity (P50) occurs. The
287 hydraulic safety margin is defined as the difference between naturally occurring minimum xylem
288 pressures and pressures that would cause 50% of hydraulic dysfunction. The naturally occurring
289 minimum xylem pressure is usually related to stomatal activity and pressure at which stomata
290 start to regulate water loss. Once a plant has reached its P50, it is on the steepest part of the
291 vulnerability curve, which means that even a small decrease in xylem pressure will produce a
292 substantial reduction of Kh (Johnson *et al.*, 2012), increasing the risk of runaway embolism
293 (Tyree & Sperry, 1988). Usually, plants with narrow safety margins experience large amounts of
294 embolism in their respective environments and, therefore, show a high risk of hydraulic failure
295 (Choat *et al.*, 2012).
296
297 Differences in hydraulic safety strategies are reported between angiosperms and conifers.
298 Conifers tend to have greater safety margins than angiosperms (Choat *et al.*, 2012, Meinzer *et*
299 *al.*, 2009) and also experience smaller levels of embolism in the stem while being more
300 permissive of embolism formation in distal organs (i.e. leaves and roots), (Johnson *et al.*, 2012).
301 This strategy can be considered as a safety valve to protect the integrity of the stem hydraulic
302 pathway as it is impossible to replace the main stem while branches and roots can be regenerated
303 or potentially refilled (McCulloh *et al.*, 2011). Angiosperms are characterized by narrow or even
304 negative safety margins making them vulnerable to extensive levels of embolism formation
305 across branches and stems. Plants have evolved mechanisms to restore xylem function (i.e.

306 embolism recovery) following events of drought (Johnson *et al.*, 2012). Safety margins of 226
307 woody species among angiosperms and gymnosperms sampled from sites with a wide range of
308 mean annual precipitation and temperature were recently compared in a meta-analysis (Choat *et*
309 *al.*, 2012). Of all species analyzed, 70% were considered to operate at narrow safety margins
310 regardless of climate preferences. However, it is important to mention here that this analysis was
311 exclusively done on small branches that are known to be more vulnerable to embolism than large
312 brunches and trunks (McCulloh *et al.*, 2014). Such a large number of species operating with
313 narrow safety margins may suggest that all biomes will be vulnerable to drought-induced decline
314 with the increases of aridity and temperature that have been predicted for many regions with
315 expected global climate change. Moreover, if extreme weather events increase in frequency and
316 severity, then drought-induced forest decline might occur not only in arid regions but also in wet
317 environments since safety margins are independent of mean annual precipitation. Thus, general
318 trends in the 'hydraulic safety margin' of trees suggest that drought-induced decline and
319 mortality at regional and global scales has the potential to occur (Choat *et al.*, 2012). Yet the
320 magnitude of this effect will depend on the ability of different species to recover from xylem
321 conductivity losses and acclimate via stomata.
322
323 Stomata provide dynamic protection from cavitation by limiting the level of negative water
324 potential that a plant experiences (Meinzer *et al.*, 1992, Sperry & Pockman, 1993). Trees adjust
325 their water use and hydraulic safety through the coordination of hydraulic and stomatal
326 regulations (Brodribb & McAdam, 2011, Choat *et al.*, 2012, Sperry, 2000). Such an association
327 between xylem water potential at the onset of xylem cavitation and leaf water potential triggering
328 stomatal closure are well documented (Brodribb & Holbrook, 2003, Brodribb *et al.*, 2003,

Cochard *et al.*, 2002, Hubbard *et al.*, 2001, Klein, 2014, Nardini *et al.*, 2001). Without stomatal control excessive embolism formation would eventually lead to 'runaway embolism', in which embolism decreases hydraulic conductance and xylem water potential, leading to a cycle of embolism that precipitously reduces water conducting capacity until it is completely lost (Tyree & Sperry, 1988). The balance between the temporal dynamics of stomatal closure and stem water potential is species-specific and is often linked to environmental properties. Under changing climatic conditions species might experience a shift in the rate of water stress enactment and, as a result, may be unable to track environmental change with stomatal closure. In fact, a sudden increase in vapor pressure deficit may lead to transient 'wrong way' stomatal response i.e. stomatal opening despite sudden drop in leaf water potential (Buckley, 2005, Buckley *et al.*, 2011) and ultimately cause a runaway embolism. However, even when stomata are capable of reducing transpiration rates, effectively protecting xylem from embolism, doing so comes at the cost of reduced photosynthetic activity and overall loss of carbohydrate reserves, which will likely be detrimental to tree health.

Tree mortality – carbohydrate starvation

Carbon starvation occurs when stomata are closed to prevent water loss and avoid hydraulic failure. During shorter timeframes, species have strategies that account for the imbalance between carbon uptake by photosynthesis and carbon loss due to respiration and growth. However, prolonged periods of stomatal closure might lead to a negative total carbon balance that depletes carbohydrates reserves and ends in tissue-level carbohydrate starvation (McDowell *et al.*, 2008, McDowell *et al.*, 2013). For some temperate angiosperm species exposed to

extreme drought conditions hydraulic failure is the first cause of tree mortality (Barigah *et al.*, 2013, Urli *et al.*, 2013). In these species the lethal xylem water potential (xylem pressure correspondent to 50% of mortality) occurred when drought had caused > 88-90% loss of stem hydraulic conductivity (Barigah *et al.*, 2013, Urli *et al.*, 2013). Thus, processes involving hydraulic impairments rather than carbohydrate impairments are considered to be more important for predicting the effects of drought on forests (Anderegg *et al.*, 2012). However, carbon and hydraulic properties cannot be considered in isolation as both are interrelated and changed during mortality of aspen during a widespread die-off event (Anderegg *et al.*, 2012). Evidence of carbon starvation–induced tree mortality is scarce, maybe because it can cause trees to die only after long exposure to relatively mild water stress conditions characterized by extended periods without positive net photosynthesis (Adams *et al.*, 2013), presumably due to the reduction of carbon uptake accompanying prolonged stomatal closure (Mitchell *et al.*, 2013, Ogasa *et al.*, 2013).

Across gymnosperms it was shown that the major cause of tree mortality is related to drought and occurs when drought causes > 50% loss of stem hydraulic conductivity (P50) (Brodribb *et al.*, 2010, Brodribb & Cochard, 2009). However, in *Pinus edulis* both hydraulic failure and carbon starvation can be independent causal factors of tree mortality. Hydraulic failure causes relatively fast tree death that is characterized by rapid decline in leaf water potential, high level loss of hydraulic conductivity, and no changes in carbohydrate reserves at death. Carbon starvation resulting from prolonged shading causes slow tree death characterized by no significant change in leaf water potential and small variations in hydraulic conductivity but significant reduction in carbohydrates reserves. Changing climatic conditions may lead to co-

occurrence of both processes, water stress and carbohydrate starvation. Thus mortality in trees currently experiencing reduction in carbohydrate availability due to shading would be accelerated by hydraulic failure and additional reduction of photosynthetic capacity due to stomatal closure (Sevanto *et al.*, 2014).

While carbohydrate starvation under drought might be a problem in current conditions, the carbohydrate balance of trees growing in elevated CO_2 and temperature, is not clear. In general, both CO_2 and temperature alter tree xylem anatomy (Domec *et al.*, 2010, Maherali & DeLucia, 2000a) and wood hydraulic properties (Phillips *et al.*, 2011) such that observed anatomical and physiological changes result in lower drought tolerance (Way *et al.*, 2013). Deciduous boreal trees, for example, showed a greater vulnerability to embolism in leaves exposed to elevated temperature compared to those grown at ambient temperatures, even though vulnerability in stems did not change between treatments (Way *et al.*, 2013). Eucalypt seedlings exposed to moderate drought stress and elevated temperatures showed reduced plant carbon accumulation and increased leaf respiration. When the same seedlings were exposed to moderate drought stress and high CO_2 concentrations, they exhibited increased carbon content and dry mass accumulation. These beneficial effects vanished, however, when drought became more severe (Duan *et al.*, 2013). Unfortunately, only a few studies have analyzed how plants respond to elevated CO_2 concentration and temperature combined (Duan *et al.*, 2013, Maherali & DeLucia, 2000a, Phillips *et al.*, 2011) and more information is needed to understand how trees will respond to drought under global change conditions.

Recovery from embolism

Plants have evolved several strategies to prevent and/or mitigate the effects of hydraulic failure due to embolism and to restore xylem transport capacity once embolism occurs (Secchi & Zwieniecki, 2012, Stiller & Sperry, 2002). Restoration of xylem capacity can be divided into two sets of strategies: (1) growth and (2) maintenance. The first group of strategies includes passive, often long-term, responses like shedding leaves or small branches followed by the growth of new shoots (shrubs). They might also include growth of new xylem and the formation of new conduits to assure continuity and capacity of the transport system under adverse conditions (Sperry *et al.*, 1987, Stiller & Sperry, 2002). The second group includes active and often relatively fast physiological responses that result in the restoration of hydraulic capacity by generating positive root pressure (often only found in small herbaceous plants) (Cochard *et al.*, 1994, Ewers *et al.*, 1997, Yang *et al.*, 2012), by stem parenchyma cell activity that results in localized embolism removal in woody plants (Brodersen & McElrone, 2013, Nardini *et al.*, 2011, Salleo *et al.*, 2004), and by strategies that require access to external water (rain, fog or snow) via leaves or buds in order to soak the xylem. The last process has been observed primarily in coniferous species (Laur & Hacke, 2014, Mayr *et al.*, 2014).

The impact of climate change on the first group of strategies would be related to changes in plant growth and would depend on relative changes in carbon fixation and respiration rates and acclimation pattern observed for specific species. Specifically, it is expected that small increases in carbon assimilation due to higher CO_2 concentrations might be offset by increased respiration due to rising average temperatures, suggesting that carbohydrates available for growth

(construction) might not change or could even decrease. Thus, the potential to restore hydraulic capacity by conduit replacement, or growing new branches might not keep up with expected losses due to increased rates of embolism formation, effectively leading to increased stress experienced by plants, along with a reduction in gas exchange. Lower gas exchange would further impact a plant's ability to maintain an adequate pool of carbohydrates to replace lost conduits and branches, effectively creating a feed-forward loop that would result in progressive loss of tree vitality, growth rate and a potential yield reduction, as a higher fraction of available resources will be used for construction and maintenance. As the recovery strategies from this first group depend on plant growth and are slow, the effects of climate change in plants relying on these strategies would be expected to occur over extended periods of time (years). The effects of climate change will most likely be reflected in reduced tree crown size due to die-back of branches and in reduced total leaf area resulting in less overall transpiration. This reduction in crown size would match transport capacity of the stem but it would reduce tree vitality and might be related to increased mortality. Although, studies of the long term effect of increased temperature on respiration suggest that some plants can acclimate and respiration can be greatly reduced in higher temperatures (Atkin *et al.*, 2005, Atkin & Tjoelker, 2003). This may still lead to higher respiration rates in warmer environments than in cooler ones, but not to the degree implied by short-term temperature changes.

The second group of strategies that might allow trees to recover from xylem hydraulic capacity losses depends on the use of energy to dynamically repair embolized conduits under the presence of adverse conditions, like tension or transpiration. Such strategies potentially allow plants greater

flexibility in response to periods of drought and mediate temporal losses to photosynthetic capacity. Reconciliation of embolism recovery with continued xylem tension has proven to be difficult to understand (Holbrook & Zwieniecki, 1999, Tyree *et al.*, 1999), and only recently has *in vivo* imaging provided strong support for the notion that plants can recover embolized vessels, even under low or moderate stress levels (Brodersen *et al.*, 2010, Clearwater & Goldstein, 2005, Zwieniecki *et al.*, 2013). However, despite significant scientific efforts (Nardini *et al.*, 2011, Salleo *et al.*, 1996, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011, Zwieniecki & Holbrook, 2009), the mechanisms responsible for embolism recovery under negative pressure is still not well understood.

Predicting future climate impact on the embolism recovery process is difficult because the specific physiological mechanisms involved are still unknown, however, a more general look at the impact of climate change on embolism recovery is possible.

While embolism formation is a spontaneous, purely physical process related to the degree of tension in the water column and to the physicochemical and anatomical properties of the wood (Brenner, 1995, Tyree & Zimmermann, 2002), embolism removal requires that empty vessels fill with water against existing energy gradients. Thus, recovery from embolism cannot happen spontaneously and requires physiological activity that promotes water flow into embolized vessels (Holbrook & Zwieniecki, 1999, Salleo *et al.*, 2004, Secchi *et al.*, 2011, Tyree *et al.*, 1999, Zwieniecki & Holbrook, 2009). Visual evidence from cryo-SEM studies, magnetic resonance imagining (MRI) observations, and computed tomography (CT)-scans showed that water (xylem sap) can return to empty vessels, suggesting that plants have the ability to restore functionality in the xylem (Clearwater & Goldstein, 2005, Holbrook *et al.*, 2001, Scheenen *et al.*, 2007). A more

recent study showed that in grapevine vessels, where the bulk xylem tissue was still under tension, water droplets preferentially formed on the vessel walls adjacent to parenchyma cells and that these droplets grow until the lumen was completely refilled (Brodersen *et al.*, 2010), although it was not proven that these refilled lumens returned to full function i.e. being able to transport water under tension. Droplet formation on the walls of empty vessels that are in contact with parenchyma cells support predictions that these living cells play a critical role in embolism removal by supplying energy, and possibly water, to drive the restoration of xylem hydraulic function (Brodersen & McElrone, 2013). If xylem parenchyma cells supply a significant fraction of water required for filling embolized vessels, water must pass a cellular membrane and, thus, the flow must be facilitated by aquaporins (membrane proteins that facilitate water transport across the cellular membrane). Studies on walnut (*Juglans regia*) showed that higher expression of two aquaporin proteins (JrPIP2.1 and JrPIP2.2) was observed in vessel-associated parenchyma cells at the same time that recovery from embolism took place (Sakr *et al.*, 2003). Moreover, expression levels of several PIP1 and PIP2 genes were shown to increase during the recovery process in two other species *Populus trichocarpa* and *Vitis vinifera* (Kaldenhoff *et al.*, 2008, Perrone *et al.*, 2012, Secchi *et al.*, 2011, Secchi & Zwieniecki, 2010). Detailed analysis of how the transcriptome responds to the presence of embolism in *P. trichocarpa* and *V. vinifera* petioles revealed that several aquaporin subfamilies were strongly up-regulated during refilling (Perrone *et al.*, 2012, Secchi *et al.*, 2011) especially PIP1, PIP2, and TIP.

Recovery from embolism requires a source of water to fill the empty vessels and a source of energy to overcome existing energy gradients acting against water flow. Living parenchyma cells can, parsimoniously, provide both. This assumption is supported by the inhibition of recovery in

response to either physical damage to phloem or metabolic inhibition of living cells in stems (Bucci *et al.*, 2003, Salleo *et al.*, 2004, Zwieniecki *et al.*, 2004). A series of models have proposed that sugars needed as osmoticum for refilling can be unloaded into the embolized vessels from ray parenchyma cells. Sugars can either be transported from the phloem or be released from starch stored in the stem. Once in the apoplast they can create an osmotic gradient that drives water from the parenchyma to the empty vessels (Nardini *et al.*, 2011, Secchi & Zwieniecki, 2011, Zwieniecki & Holbrook, 2009). For this process to work, the presence of embolism should alter carbohydrate metabolism and carbon partitioning between starch and soluble sugars in the xylem parenchyma and potentially influence both enzyme activities and gene expression. Indeed, both visualization techniques and enzymatic analysis of non-structural carbohydrates levels in xylem of *L. nobilis* and *P. trichocarpa* experiencing high levels of embolism demonstrated that starch content in stem parenchyma cells decreased and that soluble sugar levels increased (Nardini *et al.*, 2011, Regier *et al.*, 2009, Salleo *et al.*, 2009, Secchi & Zwieniecki, 2011). Furthermore, a drop in starch content was associated with changes in gene expression, especially down-regulation of the monosaccharide metabolic pathway (including enzymes involved in starch synthesis) and strong up-regulation of the disaccharide metabolic pathway that also includes starch degradation enzymes like alpha- and beta-amylases in the stems of *P. trichocarpa* (Secchi & Zwieniecki, 2011). The same study also revealed an up-regulation of genes from ion transport and carbon metabolism ontology groups. Similarly, an up-regulation of carbon metabolism was exhibited during petiole recovery in grapevine (Perrone *et al.*, 2012). This transcription level response matched results obtained from chemical analysis of liquid collected from non-functional (embolized) vessels, where elevated levels of sugars and ions were found. However, the total osmotic concentration increase was relatively small and could only account for recovery under low tension levels (Secchi

513 & Zwieniecki, 2012), thus, a significant relief from stress is most likely required before functional
514 recovery from embolism and restoration of hydraulic activity can occur.
515
516 A recent evaluation of the role of aquaporins in recovery of trees from embolism using transgenic
517 plants with down-regulated expression of the PIP1 subfamily (Secchi & Zwieniecki, 2014) in
518 combination with visual observation of the recovery process (Brodersen *et al.*, 2010, Zwieniecki *et*
519 *al.*, 2013) may suggest a new view of the embolism-recovery cycle. It is possible that embolism
520 level is a net effect of embolism formation (with a rate that is positively related to stress level) and
521 recovery (refilling, with a rate that is inversely related to stress level). This notion would explain
522 the observed higher vulnerability to embolism in the transformed plants even though the plants
523 show no anatomical changes that might affect embolism rate (Secchi & Zwieniecki, 2014): the
524 shift in vulnerability would be achieved not by any change in the rate of embolism initiation, but
525 rather by a differential rate of embolism recovery due to a lower membrane permeability of xylem
526 parenchyma cells. This view of the embolism-recovery cycle might suggest that a vulnerability
527 curve estimated from fresh material collected in the field reflects the current balance between
528 embolism and recovery processes, rather than a true measure of xylem embolism vulnerability.
529
530 Identifying how xylem recovers after embolism formation is fundamental to understanding the
531 impact of climate change on water transport and tree survival. As described above, xylem
532 recovery is highly variable among tree species and even among individual plants of the same
533 species, as recovery might depend on the current level of embolism and health status of the plant
534 (Hacke & Sperry, 2003, Holbrook *et al.*, 2001). For example, angiosperms are shown to recover
535 from embolism within days or even hours if provided relief from stress (Bucci *et al.*, 2003, Ogasa

536 *et al.*, 2013, Salleo *et al.*, 2004) while conifers tend to show very slow recovery (days or even
537 months). Such slow recovery would suggest that embolized tracheids either are refilling very
538 slowly or must be replaced by new tracheid in order to restore xylem hydraulic capacity (Brodribb
539 *et al.*, 2010, Brodribb & Cochard, 2009). However, some evidence of the fast recovery process in
540 gymnosperms has been recently shown in Norway spruce trees (*Picea abies*) that were able to
541 recover embolized xylem in later winter using melting snow as the source of water (Mayr *et al.*,
542 2014). This process was shown to be related to starch metabolism and an increase of aquaporin
543 abundance. Similarly, the role of aquaporins in facilitating radial water movement from the needle
544 epidermis towards the vascular tissue was also found in *Picea glauca* suggesting that if exposed to
545 reduced stress and foliar availability of water even conifers may have evolved a physiological
546 strategy to increase rate of recovery from embolism with refilling occuring in a matter of hours
547 (Laur & Hacke, 2014). When compared with angiosperms, gymnosperms tend to have less
548 parenchyma cells in the xylem and lower non-structural carbohydrate abundance in their wood.
549 As the ability to rapidly remove embolism relies on the proximity of parenchyma cells to xylem
550 conduits as a source of energy and water, this might explain the need for larger safety margin in
551 conifers relative to angiosperms at comparable levels of embolism (Johnson *et al.*, 2012). The
552 role of xylem parenchyma in embolism recovery is further underlined by a study showing that
553 among 47 plant species, including angiosperms and gymnosperms, axial parenchyma was
554 commonly present in most woody angiosperm species able to recover from embolism formation.
555 The few cases where parenchyma was absent were mostly in herbaceous annual plants without
556 secondary xylem where the recovery process could only be accounted by the presence of root
557 pressure (Brodersen & McElrone, 2013).
558

559 **Xylem function in the 'new climate normal'**

560

561 We are just beginning to understand fully the complexity of water transport function in the
562 xylem--including its structure, rheology, chemistry, and physiology. Our knowledge still has
563 many gaps and only a superficial knowledge exists regarding both embolism formation and the
564 process of recovery from embolism in woody plant xylem. Yet, we face the prospect of rapid
565 changes to climatic conditions that are shorter than the lifespan of a single tree. Orchards and
566 forests growing today will experience a transition period to the 'new climate normal' while we
567 have very few tools to predict climate impact on tree growth, yield and survival, and even less
568 knowledge to inform breeding efforts aimed at mediating the negative impact of changing
569 climate. However, by scrutinizing available knowledge we can filter out a few important aspects
570 of how climate change may impact xylem function and try to predict the most likely scenarios
571 describing the effect of increased temperature, reduced water availability, and increased
572 frequency and duration of extreme weather events on tree hydraulic system capacity.

573

574 We can assume that reduced water availability due to a drop in precipitation abundance,
575 frequency, or both, would lead to extended periods of time during which trees would experience
576 xylem tension levels near their 'safety margin', i.e. the level of tension when stomatal
577 conductance is reduced to protect xylem from excessive embolism formation (Figure 2). "Safety
578 margin" is an evolved trait that likely is a deeply embedded property of species-specific survival
579 strategies and is not easily changed. Thus, prolonged periods of drought that force stomata to
580 close should result in loss of photosynthetic capacity and a reduction in non-structural
581 carbohydrate availability needed by a tree to maintain transport capacity under drought and/or

582 restore it during periods of stress recovery following the relief provided by a rainfall or fog event
583 (Figure 2). Thus, under drought conditions, trees might experience an accumulation of
584 embolized conduits in stems over time, leading to reduced benefits from their evolved 'safety
585 margins'. We can expect that in the near future the potential for 'run-away cavitation' will
586 increase, especially in species operating at or near their physiological thresholds and not having a
587 strong stomatal control. In addition, if embolism level in a plant reflects the balance between
588 embolism formation rate and the capacity to refill embolized vessels using available energy, then
589 prolonged periods of drought would shift that balance toward trees capable of withstanding
590 higher levels of embolism. Under low water potential, the rate of new embolism formation will
591 exceed the rate of recovery. As frequency and length of stress relief periods are expected to
592 diminish, the recovery process will be shortened as well, forcing trees to operate at continuously
593 higher level of embolism. Operating at higher level of embolism will reduce the capacity of
594 trees to photosynthesize, as higher stress levels will force stomatal closure due to higher stem
595 hydraulic resistance. This general reduction in carbon assimilation will reduce the total
596 carbohydrate pool that could affect all aspects of plant growth, protection, and yield. This
597 reduction in production will be magnified by rising average temperatures that would increase
598 plant respiration which will further decrease the size of the total carbohydrate pool and the
599 ability of trees to maintain xylem transport capacity. Such significant reductions in the stem
600 non-structural carbohydrate pool would also lead to reduced growth, higher rates of mortality,
601 and may reduce the yield of commodity trees.
602 In conclusion, mediating future climate impacts and guiding breeding programs will require
603 better knowledge of tree stress physiology. Specifically, we should invest in improving our
604 understanding of xylem physiology under variable levels of stress. We are missing basic

information regarding daily carbohydrate balance, structure of carbohydrate use, and plant flexibility to shift energy among multiple competing demands like maintenance, growth, stress response, and recovery reserves. We are also missing fundamental knowledge regarding the biological significance of how observed embolism/recovery relates to basic phenomena at both cellular and whole plant levels. Without this basic understanding, we cannot confidently predict how trees will respond to the 'new climate normal'.

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Figure legends

Figure 1

Embolism formation in a vessel may originate from multiple sources including (1) breaking of the air/water interface at the bordered pit membrane if an adjacent vessel was already embolized, (2) expansion of air pockets from vessel crevices or (3) expansion of persistent air bubbles on the wall surface that could be remnants of previous embolism. The air-water interface might be in equilibrium if its surface tension is sufficient to oppose the tension from water in the transpiration stream. If tension from water in the transpiration stream exceeds water surface tension the bubble will expand. Such an expanding bubble will continuously decrease its radius if water/wall contact is fixed in one position. If tension in the vessel exceeds the maximum tension sustained by the bubble (at critical radius), the air-water interface breaks and the bubble expands forming an embolism.

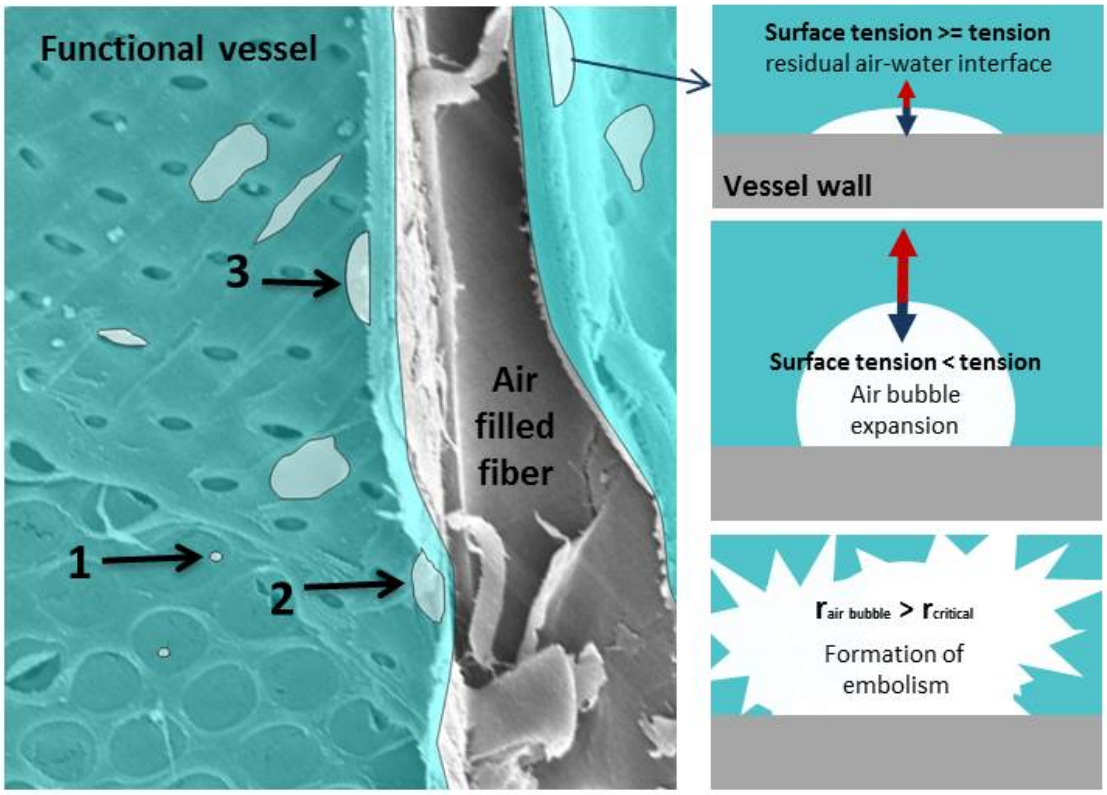
Figure 2

Figure 2

Xylem transport capacity depends on the number of functional vessels (I) that can transport water under tension. The number of functional vessels is the total number of vessels in the stem minus embolized vessels and those refilled but not yet functionally linked to vessels conducting water under tension. Rates of embolism formation (II) and refilling (III) determine the number of non-functional vessels while the rate of returning vessels to functional state (IV) determine number of vessel capable of sustaining tension (V). These rates are under physiological control that will be impacted by climate change. Specifically, increasing atmospheric vapor pressure

deficit (VPD) may result in increasing transpiration rates (a) that in turn would increase water tension in the xylem (b). Increasing xylem water tension would increase the rate of embolism formation (c) and effectively the number of embolized vessels. High tension would also negatively impact both potential for refilling of vessels (d) and rate of returning vessels to a functional state (e). In addition, climatic changes are expected to affect solar radiation and temperature which would have a major impact on both photosynthesis and respiration rates (f). Respiration rates are expected to increase with increasing temperature while photosynthesis rates are expected decrease due to both higher temperature and stomatal closure resulting from higher tension levels (g). Together such an impact would reduce the level of non-structural carbohydrates stored in the stem that would be available to provide energy required for vessel refilling (h), thus reducing the rate of refilling (i) and returning vessels to a functional state (k).

1037 Figure 1



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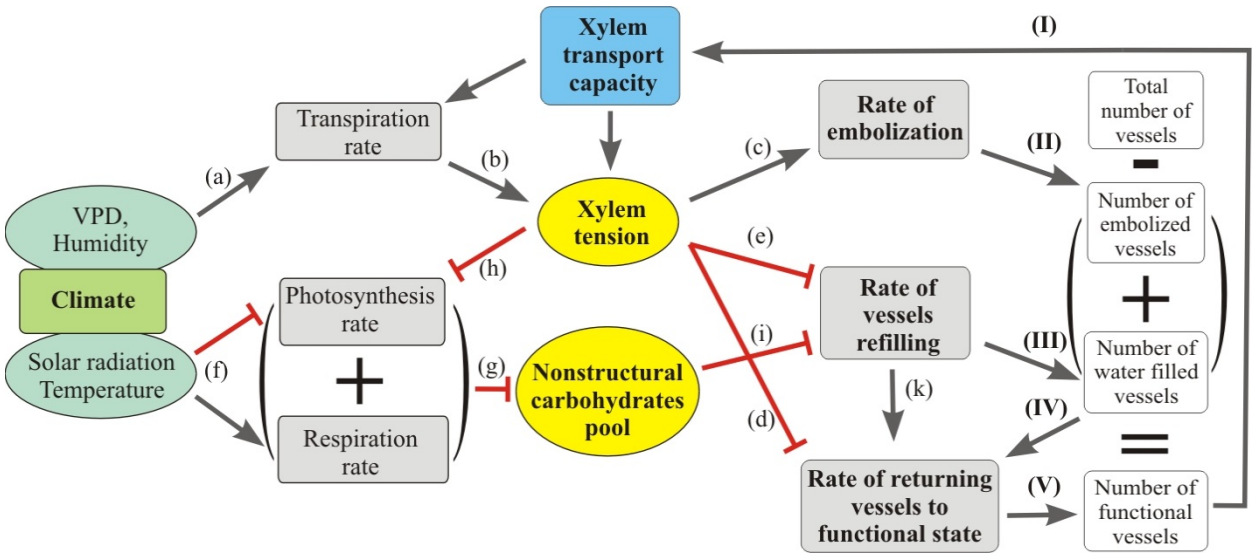
1041 Figure 2



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